RAMAN CHEMICAL IMAGING OF HUMAN BREAST TISSUES

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Chemical Imaging combines molecular spectroscopy and digital imaging to provide molecular images that detail sample morphology, composition and structure. The development of the FALCON Raman Chemical Imaging microscope (ChemIcon Inc.) allows spatial resolution of 250nm, and has a demonstrated spectral resolving power of less than 0.1 cm-1. This allows the chemical imaging and Raman spectral analyses of microscopically important features including microcalcifications and their associated protein matrices, basement membrane composition, and the content of secretory vesicles, among other important subcellular structures. In the process of evaluating a spectrum of breast lesions from simple cystic duct dilation, through hyperplasias, to invasive carcinomas, various methods of preparing the slides (background media) were evaluated. Different tissue fixations and processing procedures were compared. Examination of the tissues were carried out using 532nm and 790nm laser excitation wavelengths, respectively. Various supporting media were used including aluminum slides, optical glass slides, and fused quartz slides. A combinaiton of background control techniques, signal filters, and the fused quartz substrate gave the most reprodicible data with the best signal to noise ratios. Tissue integrity was maintained with both systems although prolonged exposure (> 5 minutes) resulted in some tissue loss using 1.5 watts with the 790nm laser source. Spectral processing (divide, zap, baseline, smooth, normalize) was accomplished with ChemImageä software (ChemIcon Inc.). Evaluation of contributions from epithelial, stromal, and fatty tissue components were evaluated in the range 1200cm-1 to 1800cm-1. Tissue section thickness of 5, 10, 15, and 20 microns were tried. While the 20 micron sections gave the highest signal to noise ratio spectra, the morphology with this thickness was less readily interpreted. Examination of calcifications associated with benign, dysplastic, and malignant lesions demonstrated differences in the associated proteins between these classes of lesions. The use of high resolution molecular spectroscopy combined with high resolution digital imaging provides a unique tool to explore spatial and chemical features of human breast lesions.

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